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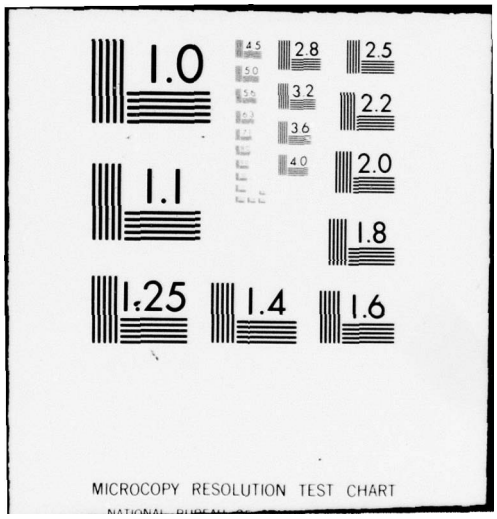
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REPORT NO. CGD-27-79

LEVEL II

EVALUATION OF THE EFFECTIVENESS OF BIOLOGICAL DEFLECTORS

FOR DEFLECTING FISH FROM ENTRAINMENT IN ICE WATER COLUMN

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U.S. Coast Guard Research and Development Center
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FINAL REPORT

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WASHINGTON D.C. 20590

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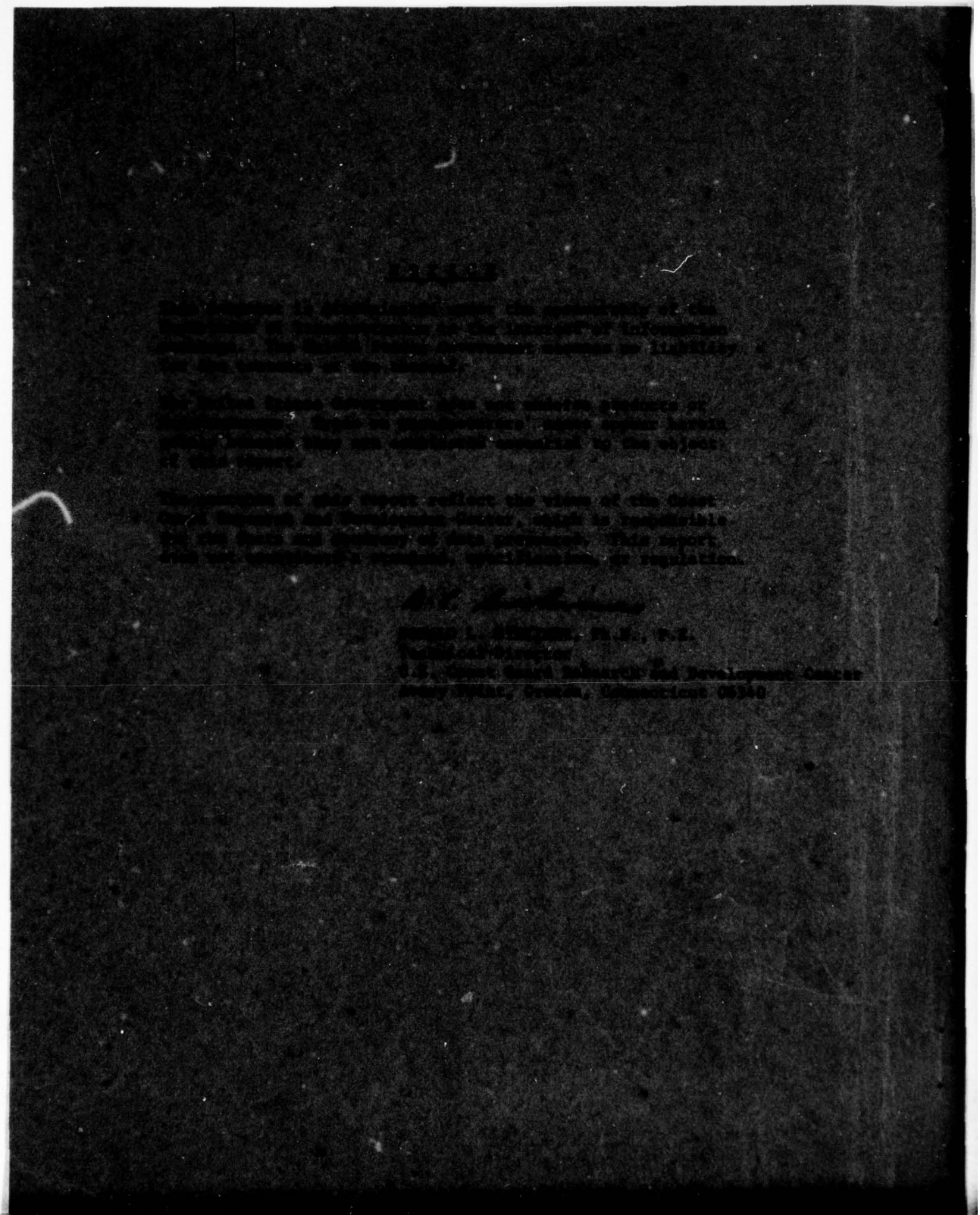

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16. Abstract

The objective of this report was to determine the utility of bio-accumulators in a line monitoring program and in assessing an oil spill cleanup by examining data obtained from the exposure of select marine organisms to crude oils in laboratory and field studies designed to simulate real world conditions.

Oysters, mussels, crabs, and marshgrass were used in the laboratory studies and were subjected to oil in flow-through fiberglass tanks. Field studies were conducted in estuarine-ponds that were subjected to salinity changes. The various biological organisms were exposed in the ponds to different concentrations of oil over extended periods of time before analysis.

The results of these studies do not support the use of biological monitors as the primary means of detecting petroleum hydrocarbon levels in the water column in the vicinity of a proposed deep water port site or following an oil spill cleanup.

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C. Douglas /Minchew
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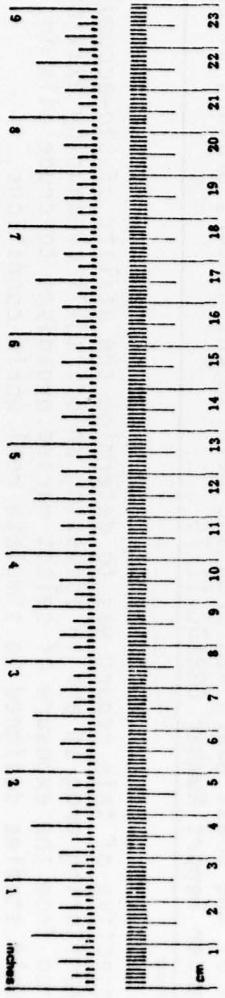
METRIC CONVERSION FACTORS

Approximate Conversions to Metric Measures

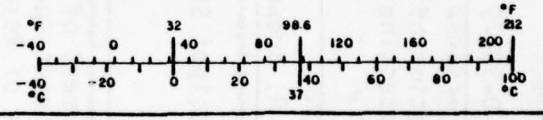
Symbol	When You Know	Multiply by	To Find	Symbol
	LENGTH			
in	inches	*2.5	centimeters	cm
ft	feet	30	centimeters	cm
yd	yards	0.9	meters	m
mi	miles	1.6	kilometers	km
	AREA			
in^2	square inches	6.5	square centimeters	cm^2
ft^2	square feet	0.09	square meters	m^2
yd^2	square yards	0.8	square meters	m^2
mi^2	square miles	2.6	square kilometers	km^2
	acres	0.4	hectares	ha
	MASS (weight)			
oz	ounces	28	grams	g
lb	pounds	0.45	kilograms	kg
	short tons (2000 lb)	0.9	tonnes	t
	VOLUME			
tsp	teaspoons	5	milliliters	ml
Tbsp	tablespoons	15	milliliters	ml
fl oz	fluid ounces	30	milliliters	ml
c	cups	0.24	liters	l
pt	pints	0.47	liters	l
qt	quarts	0.95	liters	l
gal	gallons	3.8	liters	l
ft^3	cubic feet	0.03	cubic meters	m^3
yd^3	cubic yards	0.76	cubic meters	m^3
	TEMPERATURE (exact)			
°F	Fahrenheit temperature	5/9 (after subtracting 32)	Celsius temperature	°C

*1 in = 2.54 (exactly). For other exact conversions and more detailed tables, see NBS Misc. Publ. 286, Units of Weights and Measures, Price $2.25, SD Catalog No. C13.10:286.



Approximate Conversions from Metric Measures

Symbol	When You Know	Multiply by	To Find	Symbol
	LENGTH			
mm	millimeters	0.04	inches	in
cm	centimeters	0.4	inches	in
m	meters	3.3	feet	ft
m	meters	1.1	yards	yd
km	kilometers	0.6	miles	mi
	AREA			
cm^2	square centimeters	0.16	square inches	in^2
m^2	square meters	1.2	square yards	yd^2
km^2	square kilometers	0.4	square miles	mi^2
ha	hectares (10,000 m^2)	2.5	acres	
	MASS (weight)			
g	grams	0.035	ounces	oz
kg	kilograms	2.2	pounds	lb
t	tonnes (1000 kg)	1.1	short tons	
	VOLUME			
ml	milliliters	0.03	fluid ounces	fl oz
l	liters	2.1	pints	pt
l	liters	1.06	quarts	qt
l	liters	0.26	gallons	gal
m^3	cubic meters	35	cubic feet	ft^3
m^3	cubic meters	1.3	cubic yards	yd^3
	TEMPERATURE (exact)			
°C	Celsius temperature	9/5 (then add 32)	Fahrenheit temperature	°F



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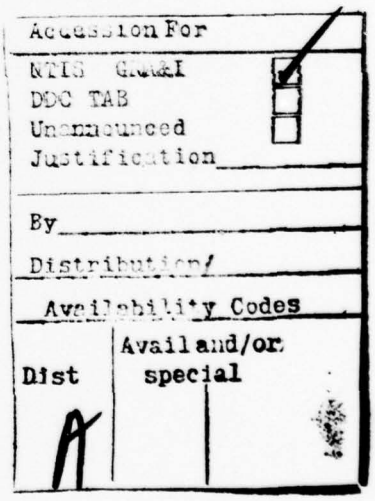

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INTRODUCTION

This study was undertaken to determine to what extent biological specimens could be used to accurately assess oil spill cleanups and to assess their potential for use in a line monitoring program in the vicinity of the proposed deep water port (DWP) sites.

The two most plausible approaches are the use of biological specimens which have the ability to concentrate petroleum hydrocarbons and the establishment of a monitoring program designed to detect shifts in resident populations towards indicator species whose population densities are favored by the presence of oil.

The indicator species concept was ruled out since it would require that the site be exposed to a level of oil sufficient to cause population shifts towards an oil-tolerant organism and away from an oil sensitive organism. This impact on the resident population is contrary to the RFP which required that any potential monitoring system must be able to detect oil at levels below those which would cause biological harm. Such a program, however, would have a place in long range studies designed to document potential impacts of the DWP sites on local marine communities.

The use of organisms with the ability to concentrate petroleum hydrocarbons from water containing low levels of oil appear to be the only practical approach to establishing a biological monitoring system. These animals could be placed at strategic places in the vicinity of each DWP site and monitored on a routine basis. This approach would be especially attractive if a chemical alternative could not be found. There is no doubt that information about the presence or absence of oil could be obtained using this method. There is, however, some question as to how the data obtained from such a monitoring program would relate to actual fluctuations of oil-related hydrocarbons. In other words, does there exist an easily interpreted, predictable relationship between the level of exposure, the time and duration of exposure, and the tissue level of petroleum hydrocarbons present in samples taken for analysis

It is, therefore, the intent of this report to examine the utility

of bioaccumulators in a line monitoring program and in assessing an oil spill cleanup by examining data obtained from the exposure of selected marine organisms to crude oils under laboratory and field conditions.

MATERIALS AND METHODS

ANALYTICAL METHODS

All oil analyses were conducted using a Waters Associates ALC/GPC-502 liquid chromatograph with an FS-770 Schoeffel fluorometer. A 30 cm x 6 nm OD, 10 μ Porasil column was used with a chloroform solvent flow rate of 1.0 ml/min. All sample preparations and analyses were conducted according to the methods reported by Miles, et. al., 1977.

LABORATORY STUDIES

Test Animals and Materials

Oysters (Crassotrea virginica), mussels (Modiolus) crabs (Calliplectes capidus), and marshgrass (Juncus roemerianus) used in the laboratory studies were obtained from the Gulf Coast near Ocean Springs and Bay St. Louis, Mississippi.

The oysters and mussels were obtained fresh, transported back to Mississippi State in burlap bags, and maintained in vats containing artificial seawater.

The crabs were captured in the wild and transported back to the laboratory in large styrofoam ice chests containing a small amount of water to aid in maintaining moisture. They were held in the laboratory as described for the oysters and mussels.

During hot weather, specimens were transported to the laboratory in an air-conditioned van to minimize temperature realted stress and mortality.

Artificial seawater (15 o/oo) made with Rila Salts Mix and non-chlorinated well water was used for holding animals and in all experiments.

The marshgrass was cut and brought back to the laboratory for drying prior to use.

Test System Design

All laboratory experiments discussed in this report were conducted in the flow system illustrated in Figure 1. It is of a closed recirculating design which allowed large volumes of water to be pumped from

under an oil slick into a mixing tank, then simultaneously passed through individual test chambers, and finally returned underneath the oil slick with a minimum disturbance of the surface. A description of the system follows. This system was composed of two 190 gallon fiberglass tanks (84"L x 22"W x 24"D) which were stacked over each other as illustrated in Figure 1. Oil was spilled on the surface of the bottom tank. Two submersible pumps (all pumps used were Teal model 1P598 submersible recirculating pumps) were placed just above the bottom at each end of the tank to circulate the water underneath the oil slick thereby aiding in producing a relatively uniform mixture. Water was pumped from under the oil slick using 5 additional pumps (placed on the bottom of the tank) via 3/8" tygon tubing into the upper mixing tank. Two pumps were used for mixing in this tank as in the lower tank. This upper tank was fitted with a 2" overflow pipe which delivered excess water back along the bottom of the oil tank. Water from the mixing tank was distributed to the test chambers in the wooden vat via individual siphons constructed of 3/8" ID glass tubing and then removed from the test chambers via 1¼" standing pipes (PVC) which extended beneath the surface of the oil tank.

The system was constructed with safety features incorporated into the design to prevent overflow of oiled water from the system in the event of pump failure. At the beginning of each test, the water level in the oil tank was adjusted so that the stand pipe extended 3" above the water surface. A sufficient number of pumps was used to produce an excess of water in the mixing tank. Therefore, water was continually returned from the mixing tank to the oil tank via the overflow pipe. The length of the mixing tank end of each siphon was cut so that it extended 2" beneath the water surface. In the event of a power failure, the siphons would break after draining 2" of water off the mixing tank. This additional water would leave the oil tank 1" away from overflow when the system had stopped circulating.

Field Studies

The field portion of this study was conducted in a pilot-plant ecosystem consisting of 4 estuarine-ponds measuring 30.5 x 30.5 x 2.8 M which were constructed at the NASA National Space Technology Laboratory (NSTL) in Hancock County, Mississippi. The ponds had been previously

filled with seawater (28 o/oo) barged in from the Gulf of Mexico (Brown, 1975). The final salinities were adjusted to approximately 14 o/oo with fresh well water. Clumps of Juncus roemerianus, mixed occasionally with other species (e.g., Spartina, Scirpus, and Distichlis), were dug from selected areas in the St. Louis Bay marsh (Hancock County, Mississippi), transported to NSTL, and immediately planted along the banks of each of the ponds. The clumps included the natural muddy substrate along with its associated benthic fauna and microflora. These estuarine-pond ecosystems became established during the first year following their construction as evidenced by the development of a rich organic sediment, by the continual growth and regeneration of the marsh plants, and by the responsiveness of the plankton populations to salinity changes. During periods of high salinities (10-12 o/oo), the planktonic populations were predominantly marine, while during periods of low salinities (5-6 o/oo) they were predominantly freshwater and/or euryhaline. These shifts in the plankton populations were encouraging because they indicated that the planktonic populations in the ponds were responding to salinity changes in a manner similar to those in natural marshes.

The salinity in the ponds had dropped to 5-6 o/oo prior to the initiation of the present study. This deficiency was corrected with the addition of bulk quantities of commercial salts. The amount of each to be added was determined by calculating the amounts of Na, K, Ca, Mg, Cl, and SO_4 ions in Rila Sea Salts Mix which is routinely used in laboratory experiments requiring saltwater. The above ions were added in the form of NaCl (14,000 lbs/pond), $CaCl_2$ (1,675 lbs/pond), $MgSo_4$ (3,300 lbs/pond), KCl (350 lbs/pond), and $MgCl_2$ (2,424 lbs/pond). In all, a total of 87,000 lbs of salts were added to the system.

In late March of 1978, each pond was stocked with 400 oysters. They were placed in baskets which were suspended approximately 1 foot above the bottom in each corner of the respective ponds.

Each of the four ponds was divided into 16 equal quadrants. The quadrants were marked using nylon line stretched across the ponds and tied to stakes placed at the appropriate points on the banks. During each sampling, a total of 20 water and sediment samples were taken from each pond. A water sample was taken 1 foot beneath the surface in each of 16 quadrants. Four additional samples were taken approximately 1

foot above the bottom in the four center quadrants. In addition, 1 sample was taken at the waterline on each of the 4 sides of the respective ponds. A total of 5 oysters were taken from the corner of each pond during each sampling.

Water and mud samples were placed in glass containers and kept on ice during transport back to the laboratory for analysis. Live oyster samples were placed in bags for transport back to the laboratory where they were shucked and analyzed.

RESULTS

Laboratory Studies

All laboratory studies undertaken in support of this task were conducted in the flow system illustrated in Figure 1. The flow system was designed to allow a large number of animals (or materials) to be exposed to water drawn from under a known amount of oil. The size of the system also allowed a total of 11 water samples (1 liter each) to be withdrawn during each sampling and replaced by an equal volume of clean water without disrupting the system.

Preliminary experiments were conducted to determine if the system functioned as expected.

In the first experiment, 25 ppm Empire Mix crude oil was spilled on the oil reservoir tank. One liter water samples were taken from the bottom of the oil reservoir and mixing tanks (2 at opposite ends), and from the test compartments (one/compartment) each day for 9 days (216 hours).

As can be seen in Figure 2, oil was not detected during the first 24 hours. During the next 24 hours, the concentration of oil, rose sharply and then began to decline. At approximately 96 hours, the concentration of oil in all tanks leveled off indicating that a state of equilibrium had been achieved. The concentration of oil in the water samples from all tanks again began to rise sharply between 192 and 216 hours. This second rise was attributed to the decrease in the water level in the oil reservoir tank caused by the removal of water samples for analyses. This low water level allowed the oil, which until this time had remained undisturbed on the surface, to be sucked up by the pumps and transferred into the system.

A second preliminary experiment was conducted to determine the extent to which oysters and mussels could concentrate petroleum hydrocarbons from the water column when present at concentrations below the detectable limit of the LC-fluorescence method.

Empire Mix oil (25 ppm) was spilled on the oil reservoir tank and water samples were taken for analysis as before. When oil was no longer detectable in the water column, oysters and mussels were placed in test

compartments as follows: 5 oysters and mussels in compartments 1, 2, and 3; 10 oysters in compartments 4 and 6 and 10 mussels in compartments 5 and 7. After 96 hours, all organisms were removed and analyzed for oil. Thirty-three of the 35 oysters contained oil concentrations ranging from 0.27 to 5.34 µg/g, while 11 out of 35 mussels contained oil concentrations ranging from 0.03 to 3.29 µg/g (Table 1). Clearly, under the conditions of the test, the oysters were the superior concentrators. Therefore, they were selected as the principle test organism for all future experiments.

In the final preliminary test, ten oysters were placed in each test compartment. Empire Mix crude oil (25 ppm calculated) was spilled on the oil reservoir tank. Water samples (2/tank; 1/test compartment) and oyster samples (1/test compartment) were taken daily for 6 days. The water removed for analysis was replaced by an equal volume of clean seawater.

The levels of oil detected in the water column are given in Table 2. Unexpectedly, the levels of oil were still high after 144 hours. A close examination of the system indicated that this was due to the emulsifying action of detergent inadvertently left in the system. The test was discontinued once the problem was discovered.

All but one of the oysters examined prior to termination of the test contained concentrations of oil ranging from a low of 0.24 µg/g to a high of 27.83 µg/g (Table 3). The concentration of oil present in the tissues of these animals was much higher than the levels found in animals from the previous experiment. Again, as with the water, these higher concentrations were attributable to the action of the detergent.

The results of the preliminary experiments indicated that the basic design of the system was good. A number of procedural changes such as replacement of the water removed for analysis, relocation of several pumps to improve mixing and implementation of a much more rigid cleanup routine eliminated the minor problems encountered earlier.

In the first major experiment, the flow system was filled with 10 o/oo salt water (1127.42 liters). Ten marked oysters were placed in each of the 7 test compartments. Following a 24-hour equilibration period, 25 ppm (calculated) Empire Mix crude oil was spilled on the oil reservoir

tank. Eleven one liter water samples and 7 oyster samples (one/compartment) were removed each day for 15 days. Oysters removed for analysis were replaced in order to maintain a relatively constant biomass. The results of this experiment (Figure 3) indicated that the levels of oil in the oysters did not accurately reflect the sharp rise and decline of oil in the water column during the first 240 days. The oysters did have slightly higher values than were found in the water column after 264 hours.

At the conclusion of this test (360 hours) the concentration of oil in the water column was less than 1 µg/l. At this point, 2 crabs and 3 bundles of dried marshgrass were placed in separate test compartments to determine if either had the ability to concentrate the oil in significant quantities. When analyzed, the muscle tissues of the crabs contained 0.4 and 0.9 µg of oil per gram tissue, while the 3 marshgrass bundles contained 76, 142, and 103 µg of oil per gram tissue, respectively.

A second experiment was conducted using the same procedures with the exception that 75 ppm oil was used instead of 25 ppm. As indicated in Figure 4, the same basic pattern of rise and fall of water column and oyster tissue levels occurred in this experiment as in the first. The maximum levels of oil in the water column were much higher in this test (3000 µg oil/liter wet vs 200 µg oil/liter water) than in the first test and they reached their peak at about 25 hours later. The oyster tissue levels of oil were also higher in this test than in the first, but not nearly to the extent that they were in the water samples (100 µg of oil/g tissue vs. 30 µg of oil/g tissue). Another major difference was the high oyster mortality in the second experiment (there was no mortality in the first experiment). The second test was terminated after 368 hours. At that time the average concentration of oil (µg/liter) in the water column was 337 in the oil tank, 284 in the mixing tank and 304 in the test compartments.

At the conclusion of the above test, one crab was placed in each test compartment. Bundles of dried marshgrass were place in each test compartment and suspended under the surface in the oil reservoir and the mixing tanks. They were removed for analysis after 96 hours. The results of these analyses are given in Tables 4 and 5. It is clear that

the levels found in the marshgrass more closely reflect the actual levels found in the water column than did the levels found in the tissues of the crabs.

Field Studies

The field studies were conducted in estuarine ponds located at the National Space Technological Laboratory (NSTL) in Hancock County, Mississippi. The ponds were stocked and sampled as described in the Materials and Methods.

Pre-spill water and sediment samples were taken on 10 April 1978. On 12 April 1978, three of the ponds were treated with either Saudi Arabian, Nigerian, or Empire Mix crude oils at a level of 22.6 l/pond (4.0 mg/l calculated). If this oil had been evenly distributed on the surface of the ponds, it would have produced an oil slick 0.01-0.02 mm in thickness. The 4th pond was retained as a control.

The results obtained from the analysis of port-spill water samples taken after 6 hrs, 1, 2, 3, 4, 12, and 23 days are given in Table 6. These results indicate that oil rapidly entered the water column of the ponds. After 6 hrs, the average concentration of oil in the ponds was 90.3, 19.7, and 53.0 µg of oil per liter of water in the Saudi Arabian, Nigerian and Empire Mix treated ponds. After 24 hrs, the average concentration of oil in the water samples remained steady at 90.3 µg/l in the Saudi Arabian treated pond but dropped to 9.7 µg/l in the Nigerian treated pond and 18.8 in the Empire Mix treated pond. By 48 hrs the average concentrations of oil had dropped to 2.9, 0.3, and 0.5 µg/l in water from the Saudi Arabian, Nigerian and Empire Mix treated ponds respectively.

The results obtained from the analysis of post-spill sediment samples are given in Table 7. Sediment samples were not taken for the first 24 hours due to the unlikelihood that significant levels of oil would reach the bottom prior to this. In addition, the activity in the pond was restricted during the period of time that the oil was on the surface to prevent the slick from being unnecessarily disturbed. Water samples were taken in spite of this due to the likelihood that all significant

data would be lost if they were not taken.

All three ponds had low levels of residual oil from a previous study. It is interesting to note that the levels of oil in the sediments increased more in the Saudi Arabian pond than in the Nigerian and Empire Mix pond.

The results obtained from the analysis of post-spill oyster samples are given in Table 8. It is evident that the oysters contained a low level of residual oil when stocked, since many control oysters contained oil. The concentration of oil in the oysters remained relatively constant (averaging 0.4 to 2.9 µg oil/g tissue) during the first 11 days. By day 38, all oysters in this pond were dead. There was also high oyster mortality in the Nigerian pond after 38 days.

DISCUSSION

The purpose of this task was to determine to what extent biological organisms could be used in an overall monitoring program designed to detect low level petroleum contamination of waters around the proposed DWP sites and to determine their potential for use in assessing the effectiveness of an oil spill cleanup.

It is now well-documented that many marine organisms, especially mollusks, have the ability to concentrate and, given sufficient time, to depurate specific fractions of crude oils (Blumer et al., 1970; Lee et al., 1972; Stegeman et al., 1973). Therefore, the most obvious use of biological specimens would be to establish a program designed to monitor the long-term build-up of petroleum hydrocarbons at specific locations by routinely analyzing tissue levels of resident organisms. This type monitoring program would be a good barometer for indicating subtle increases in petroleum in a given ecosystem. It would not, however, allow judgements to be made about separate closely-occurring spills due to the difficulty interpreting uptake and depuration data. The animals would probably be depurating the hydrocarbons they had concentrated following exposure to oil from the first spill when exposed to the second spill.

The problem caused by separate closely-occurring inputs could be partially alleviated by placing select organisms at specific stations around the area of interest or at the sites of oil spills after cleanup procedures had been completed. Clearly, the analysis of such organisms would show whether or not they had been exposed to oil. The question then becomes: Can the tissue levels of such an organism be related to the actual levels it has been exposed to with ease and reliability? If not, their value must be questioned and the data generated from their use should be interpreted with extreme care.

The results of the present study do not support the use of biological specimens in any program except in one designed to determine the presence or absence of oil. It is obvious from the results of the laboratory studies, that there is no direct relationship between the average tissue levels of oil in oysters and the level of oil to which they were exposed. For example, spilling 25 mg of oil/liter of water on

the surface resulted in a maximum average of about 210 µg of oil per liter of water in the column and a maximum average of about 30 µg of oil per gram of tissue in exposed oysters (Figure 3), while spilling 75 mg of oil per liter of water on the surface resulted about 3000 µg of oil per liter of water and about 120 µg of oil per gram of tissue (Figure 4).

Obviously, it would be difficult to arrive at the level of exposure simply by knowing the tissue concentration at a given time. The situation is further complicated by the pattern of rapid buildup and decline in the water column and the much slower buildup and gradual decline in the oysters as illustrated in all studies. The time of exposure and the time since exposure, therefore, become very important. Information such as this would, in all probability, not be available in a routine monitoring program designed to identify inputs of crude oil which are too small to be identified visually. Even when used to assess cleanup procedures, it would be difficult to relate tissue concentrations to specific levels in the water column due to biological variations which influence the concentrating ability of specific animal. This variation was evident in all studies. Another serious problem in trying to relate tissue levels to actual exposure levels in real world situations is the need to know pumping times and pumping rates in order to be able to calculate the concentration of oil in the water.

As indicated in Table 5, use of a non-living material such as dried marshgrass offers greater potential than using living organisms which are subject to so many variations. The levels of oil absorbed by the different marshgrass samples were reasonably consistent and relatively close to actual water column levels of oil.

The results obtained from the pond study further support the conclusion that biological specimens are not superior to other methods of oil detection. There were no results obtained from the analysis of the oysters from the respective ponds which were not also obtained from the analysis of the sediment samples.

In conclusion, the use of biological monitors as the primary means of detecting petroleum hydrocarbon contamination in the vicinity of the proposed DWP sites or following cleanup procedures is not recommended

due to problems associated with their deployment, due to problems associated with subsequent interpretation of the data on a real world basis.

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Table 1. Oil concentrations in oysters and mussels exposed to 25 ppm crude oil in a close-flow system. Tissue level of oil determined using HPLC with 403 nm excitation wavelength and measuring fluorescence at 418 nm.

Tank 1		Tank 2		Tank 3		Tank 4	Tank 5	Tank 6	Tank 7
Oysters	Mussels	Oysters	Mussels	Oysters	Mussels	Oysters	Mussels	Oysters	Mussels
1.00	0.31	0.74	0.24	1.17	0.35	0.27	3.26	0	0.43
1.36	0	0	0	0.32	0	0.27	0.14	0	0
1.26	0.03	1.36	0	0.31	0	0.90	0.17	0.70	0
2.94	0	1.92	0	0.52	0	1.40	0	0.61	0
5.34	0		0	1.97		3.92	0	1.90	0.73
						2.48	0.31	3.27	0
						1.25	0	3.29	0
						0.71	0	0.45	0.05
						1.28	0	0.51	0
						2.35	0	0.71	0

Table 2. Oil concentrations in various compartments of a closed-flow system following contamination with 25 mg of oil per liter of water. Oil levels determined using HPLC with 403 nm excitation wavelength and measuring fluorescence at 418 nm.

Hours	Tank 1	Tank 2	Tank 3	Tank 4	Tank 5	Tank 6	Tank 7	Top Left	Top Right	Bottom Left	Bottom Right
Control	0*	0	0.11	0	0	0	0	0	2.35	0	0
24	126	52	96	135	79	103	87	83	119	112	114
48	42	35	18	20	79	35	55	58	77	86	123
72	206	130	132	155	148	153	147	140	115	172	102
96	115	179	242	161	319	200	115	195	248	159	299
120	129	338	163	406	394	448	195	240	121	405	176
144	167	107	213	213	229	159	194	260	125	214	419

*Values are given as μg of oil per liter of water.

Table 3. Oil concentration in oysters following exposure to 25 mg of oil/liter of water in a closed-flow system. Oil concentrations determined using HPLC with 403 nm excitation wavelength and measuring fluorescence at 418 nm.

Hours	Tank 1	Tank 2	Tank 3	Tank 4	Tank 5	Tank 6	Tank 7	Average
Control	0*	0	0	0	0	0	0	0
24	2.32	15.64	1.11	5.47	6.39	1.43	7.87	5.75
48	4.36	5.02	5.26	7.73	14.49	16.69	3.03	8.08
72	6.02	0.50	0.24	5.35	27.83	7.49	0.63	6.86
96	4.57	4.40	13.48	23.45	5.59	15.04	6.65	10.45
120	0	1.58	3.24	1.45	1.22	1.38	0.88	1.39
144	7.09	2.48	3.31	6.44	5.60	17.28	8.67	7.27

*Values are in µg of oil/liter of water.

Table 4. Petroleum hydrocarbon concentrations found in crab tissue after exposure to low levels of crude oil for 4 days in a recycling flow system. Oil concentrations determined using HPLC with 403 nm excitation wavelength and measuring fluorescence at 418 nm.

Tank Sampled	Conc. in Gill Tissue (µg/g)	Conc. in Muscle Tissue (µg/g)	Conc. in Gonad Tissue (µg/g)	Conc. in Gut Tissue (µg/g)
Oil	28.0	0.8	*	3.8
Oil	190.0	0.2	1.1	2.0
Mixing	33.0	1.9	0.3	1.5
1	10.0	0.3	8.0	3.1
2	9.0	0.4	0.5	4.3
3	4.7	0.2	0.5	1.4
4	13.0	0.3	0.3	2.3
5	9.0	0.3	0.2	0.3
6	1.1	0.1	0	3.6
7	10.0	0.2	0	0.3

*No sample.

***Concentration of oil in the water column at the time that samples were taken were: oil reservoir tank, 1158 µg/l; mixing tank, 826 µg/l; test tanks, 833 µg/l.

Table 5. Petroleum hydrocarbons distribution in marshgrass after exposure to low levels of crude oil for 4 days in a recycling flow system. Oil concentrations determined using HPLC with 403 nm excitation wavelength and measuring fluorescence at 418 nm.

Sample Number	Control Marshgrass	Oil Reservoir Marshgrass	Mixing Tank Marshgrass	Test Tanks Marshgrass
1	84*	5999	1360	916
2	2	6367	1371	705
3	<1	6640	1665	771
4	<1	5953	2549	444
5	<1			154
6				523
7				1126

*Concentration of oil in the water in g/liter.

**Concentrations of oil in the water column when samples were taken were: oil reservoir tank, 1158 µg/liter; mixing tank, 826 µg/liter and test tanks, 833 µg/liter.

Table 6. The concentration of oil in water samples obtained during the MSU/NSTL oil spill study. Concentrations are in µg/l

Date	Sample No.	Pond No.			
		2	3	4	5
4/10/78 Controls	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
	7	0	0	0	0
	8	0	0	0	0
	9	0	0	0	0
	10	0	0	0	0
	11	0	0	0	0
	12	0	0	0	0
	13	0	0	0	0
	14	0	0	0	0
	15	0	0	0	0
	16	2.3	0	0	0
	17	0	0	0	0
	18	0	0	0	0
	19	0	0	0	0
	20	0	0	0	0
4/12/78 6 hrs. post-spill	1	---	20.6	.5	508.8
	2	---	34.7	3.1	7.9
	3	---	87.0	19.4	69.6
	4	---	190.0	334.4	11.7
	5	---	83.3	1.6	136.9
	6	---	37.3	1.0	23.5
	7	---	0	0	6.4
	8	---	79.4	18.7	1.2
	9	---	932.8	0	75.1
	10	---	10.6	1.0	88.8
	11	---	44.9	9.1	19.9
	12	---	9.6	0	2.1
	13	---	67.8	.3	84.2
	14	---	0	2.2	.9
	15	---	65.3	1.9	17.6
	16	---	62.6	0	1.3
	17	---	39.9	0	0
	18	---	22.8	1.5	1.7
	19	---	15.7	.1	3.4
	20	---	1.2	0	0

Table 6. (Cont'd)

Date	Sample No.	Pond No.			
		2	3	4	5
4/13/78	1	---	15.9	4.4	23.5
24 hrs. post-	2	---	89.2	12.2	59.7
spill	3	---	86.3	37.6	21.8
	4	---	270.5	85.2	32.7
	5	---	75.6	.4	31.1
	6	---	28.5	0	28.4
	7	---	110.9	10.3	0
	8	---	72.0	6.4	41.9
	9	---	13.0	2.9	17.9
	10	---	44.9	1.0	14.9
	11	---	107.7	5.3	11.9
	12	---	71.9	16.2	.5
	13	---	159.3	0	25.6
	14	---	224.6	4.3	32.4
	15	---	237.1	.4	35.1
	16	---	2893.5	3.8	5.4
	17	---	49.2	2.2	11.8
	18	---	14.9	.6	4.5
	19	---	23.6	0	0
	20	---	20.9	.2	6.6
4/14/78	1	---	0	0	0
48 hrs. post-	2	---	0	0	.7
spill	3	---	0	0	0
	4	---	21.2	6.0	0
	5	---	0	0	0
	6	---	0	0	0
	7	---	0	0	0
	8	---	0	0	1.2
	9	---	.3	0	1.3
	10	---	0	0	3.7
	11	---	0	0	0
	12	---	0	0	0
	13	---	0	0	1.4
	14	---	0	.2	.9
	15	---	0	0	0
	16	---	0	0	0
	17	---	5.2	0	1.1
	18	---	31.8	0	.2
	19	---	0	0	0
	20	---	0	0	0

Table 6. (Cont'd)

Date	Sample No.	Pond No.			
		2	3	4	5
4/15/78	1	---	0	0	.4
72 hrs. post-	2	---	0	0	0
spill	3	---	0	0	0
	4	---	0	.2	0
	5	---	0	0	0
	6	---	0	0	0
	7	---	0	0	0
	8	---	0	0	0
	9	---	0	0	0
	10	---	0	0	.6
	11	---	0	.2	0
	12	---	0	0	0
	13	---	0	0	0
	14	---	0	0	0
	15	---	0	0	0
	16	---	0	0	0
	17	---	0	0	0
	18	---	0	0	0
	19	---	0	0	0
	20	---	0	0	0
4/16/78	1	0	0	0	0
96 hrs. post-	2	0	0	0	0
spill	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
	7	0	0	0	0
	8	0	0	0	0
	9	0	12.2	0	0
	10	0	0	0	0
	11	0	0	0	0
	12	0	0	0	0
	13	0	0	.2	0
	14	0	0	.1	0
	15	0	0	0	0
	16	0	0	0	0
	17	0	0	0	0
	18	0	0	.2	0
	19	0	0	0	.8
	20	0	0	0	1.4

Table 6. (Cont'd)

Date	Sample No.	Pond No.			
		2	3	4	5
4/24/78	1	0	0	0	0
12 days post-	2	0	38.3	0	0
spill	3	0	0	0	0
	4	0	0	0	0
	5	2.0	0	0	0
	6	0	0	0	0
	7	0	0	.1	0
	8	0	0	0	0
	9	0	0	0	0
	10	1.2	0	0	0
	11	0	0	0	0
	12	0	4.4	0	0
	13	0	0	0	0
	14	0	0	0	0
	15	0	0	0	0
	16	0	0	0	1.0
	17	0	0	3.6	0
	18	0	0	0	0
	19	0	0	0	0
	20	0	0	0	0
5/5/78	1	0	0	0	0
23 days post-	2	0	0	0	0
spill	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
	7	0	---	0	0
	8	0	0	0	0
	9	0	0	0	0
	10	0	0	0	0
	11	0	0	0	0
	12	0	0	0	0
	13	0	0	0	0
	14	0	0	0	0
	15	0	0	0	0
	16	0	0	0	0
	17	0	0	0	0
	18	0	0	0	0
	19	0	0	0	0
	20	0	0	0	0

Table 7. The concentration of oil in sediment samples obtained during the MSU/NSTL oil spill study. Concentrations are given in µg/g wet weight of sediments.

Date	Sample No.	Pond No.			
		2	3	4	5
4/9/78 Controls	1	0	18.1	.1	.5
	2	0	11.4	.1	.3
	3	0	1.4	.2	.8
	4	0	4.2	.1	.1
	5	0	.2	.1	.3
	6	0	.2	<.1	.2
	7	0	1.1	.1	.5
	8	0	.6	.1	1.0
	9	0	.1	<.1	1.0
	10	0	.2	.1	.4
	11	---	2.4	<.1	.2
	12	0	5.0	.1	.4
	13	0	1.7	.1	<.1
	14	0	.4	.1	.4
	15	0	9.7	.1	.4
	16	0	8.3	.3	.7
4/16/78 96 hrs post-spill	1	.1	3.3	.5	5.3
	2	0	1.3	.4	.2
	3	0	.6	.1	.3
	4	0	5.3	4.2	<.1
	5	0	3.7	.8	.3
	6	< .1	4.5	.1	.1
	7	0	1.7	<.1	0
	8	0	3.5	.1	0
	9	0	1.9	.1	<.1
	10	0	.9	.1	<.1
	11	0	4.1	.2	.2
	12	0	7.2	.1	<.1
	13	0	5.1	.1	1.4
	14	< .1	1.3	.1	.4
	15	0	2.5	0	.2
	16	.2	1.1	0	.1
	17	0	1.3	1.3	.7
	18	0	1.7	<.1	.7
	19	0	1.2	.3	.6
	20	< .1	34.4	.3	.3

Table 7. (Cont'd)

Date	Sample No.	Pond No. 2	3	4	5
4/24/78	1	0	4.7	.5	1.6
12 days post-	2	0	14.7	.1	.1
spill	3	0	1.6	.3	.2
	4	0	10.8	.6	.5
	5	.2	6.7	.5	.2
	6	0	8.8	.2	.5
	7	0	1.2	.3	.2
	8	---	3.2	.1	.6
	9	<.1	12.2	.1	.5
	10	0	19.1	.1	1.2
	11	0	15.5	.2	.5
	12	0	34.2	.2	1.5
	13	0	20.3	.2	<.1
	14	0	6.3	.4	.9
	15	<.1	6.5	.2	.7
	16	0	11.6	---	.2
	17	0	38.1	2.0	3.1
	18	.3	3.7	.4	2.2
	19	0	11.6	1.1	6.8
	20	.2	12.8	.3	6.4
5/5/78	1	.3	2.1	.5	.4
23 days post-	2	0	19.0	.1	<.1
spill	3	0	2.0	<.1	.4
	4	0	7.6	.9	.2
	5	<.1	4.3	<.1	.7
	6	0	9.1	<.1	.2
	7	0	4.3	---	0
	8	0	2.7	<.1	.4
	9	0	1.6	<.1	.8
	10	0	6.1	<.1	.5
	11	0	16.1	<.1	.4
	12	0	6.1	<.1	10.4
	13	<.1	2.7	<.1	1.0
	14	0	3.5	<.1	.4
	15	0	8.6	.2	.3
	16	0	5.4	.1	.7
	17	<.1	3.1	16.2	<.1
	18	0	3.0	<.1	.5
	19	0	5.6	.6	---
	20	0	23.9	.9	2.7

Table 7. (Cont'd)

Date	Sample No.	Pond No.			
		2	3	4	5
5/31/78	1	<.1	5.3	.7	3.1
49 days post-	2	<.1	4.8	.3	1.0
spill	3	0	.5	.2	1.2
	4	0	4.4	4.9	1.4
	5	0	.4	.2	1.1
	6	0	7.3	.4	.4
	7	0	2.4	<.1	.3
	8	0	4.4	<.1	.2
	9	0	.2	1.6	.1
	10	0	2.8	<.1	<.1
	11	0	.5	<.1	.1
	12	0	1.2	<.1	.4
	13	<.1	<.1	.7	1.6
	14	0	1.1	<.1	.6
	15	0	4.0	<.1	.2
	16	<.1	1.9	<.1	.5
	17	<.1	.4	.2	.4
	18	0	0	.4	1.3
	19	0	3.5	.2	11.9
	20	0	5.5	.2	1.1

Table 8. The concentration of oil in oysters obtained during the MSU/NSTL oil spill study. Concentrations are in µg/g wet weight

Days	Corner of pond	Pond No.			
		2	3	4	5
2 days	1	.3	.3	0	2.3
		1.1	0	.2	3.8
		.5	0	.6	2.2
		2.0	.8	1.5	.1
		1.9	.5	.5	.6
	2	0	25.8	.4	1.4
		.7	0	1.3	8.8
		.8	.1	.3	.9
		.5	1.3	7.0	11.6
		.3	.3	.7	1.3
	3	0	.6	1.9	0
		0	0	3	1.5
		.3	2.0	.2	.1
		0	0	0	3.8
		.9	0	0	.3
	4	0	15.3	1.7	.5
		.2	2.9	1.3	.2
		0	.7	.8	1.8
		.5	7.2	.3	4.5
		0	.3	3.4	8.6
4 days	1	0	1.0	0	.9
		.9	5.1	0	.9
		0	4.4	.4	.1
		0	.2	0	.8
		.8	0	.1	7.6
	2	0	0	.1	2.6
		.9	0	0	2.9
		.6	0	0	5.7
		.4	1.8	.3	4.5
		.3	0	.6	1.9
	3	0	2.8	1.4	1.7
		.2	0	.2	.4
		0	1.1	.1	6.4
		.6	3.5	.2	0
		.1	0	1.8	4.2
	4	.2	4.2	.4	.8
		.2	.5	.3	1.9
		.1	1.3	.2	0
		.3	.8	0	0
		.7	0	2.9	2.3

Table 8. (Cont'd)

Days	Corner of pond	Pond No.			
		2	3	4	5
11 days	1	0	1.4	2.2	0
		0	0	1.7	.4
		0	0	.7	1.2
		0	0	.1	1.8
		0	0	1.0	.4
	2	0	0	2.3	1.1
		0	.4	.9	1.1
		0	0	1.0	.5
		0	1.5	.7	0
		0	1.2	.7	.4
	3	.2	2.7	.3	3.7
		0	0	1.1	.7
		.6	1.0	2.1	1.0
		0	9.1	2.5	.1
		0	2.8	5.2	2.4
	4	0	.3	1.2	.5
		.3	.5	.6	.6
		0	1.9	.6	0
		.1	2.1	1.5	.5
		0	3.6	.4	.8
23 days	1	0	.2	.5	0
		0	0	0	0
		0	0	0	0
		0	0	.5	2.6
		0	1.1	0	0
	2	0	0	.4	.2
		0	2.3	0	.6
		0	0	0	.3
		0	0	1.3	0
		0	---	.2	0
	3	0	.3	0	.2
		0	2.5	0	0
		0	0	.6	.5
		.4	0	---	.6
		0	0	0	.8
	4	0	0	0	0
		0	0	0	.7
		0	1.5	0	.3
		0	1.1	.3	1.5
		.1	0	0	.8

Table 8. (Cont'd)

Days	Corner of pond	Pond No.			
		2	3	4	5
38 days	1	0	3.0	---	0
		0	0	---	0
		.3	.3	---	.1
		0	.1	---	.3
		1.9	3.3	---	0
	2	0	0	---	1.2
		0	3.1	---	0
		.1	---	---	.1
		0	---	---	0
		0	---	---	.1
	3	0	.3	---	0
		0	0	---	0
		0	---	---	0
		0	---	---	0
		.1	---	---	.7
	4	0	0	---	1.2
		0	0	---	0
		0	0	---	.6
		0	---	---	0
		0	---	---	0
49 days	1	---	---	.4	---
		---	---	.8	---
		---	---	---	---
		---	---	---	---
		---	---	---	---
	2	---	---	---	---
		---	---	---	---
		---	---	---	---
		---	---	---	---
		---	---	---	---
	3	---	---	---	---
		---	---	---	---
		---	---	---	---
		---	---	---	---
		---	---	---	---
	4	---	.7	---	---
		---	.8	---	---
		---	---	---	---
		---	---	---	---
		---	---	---	---

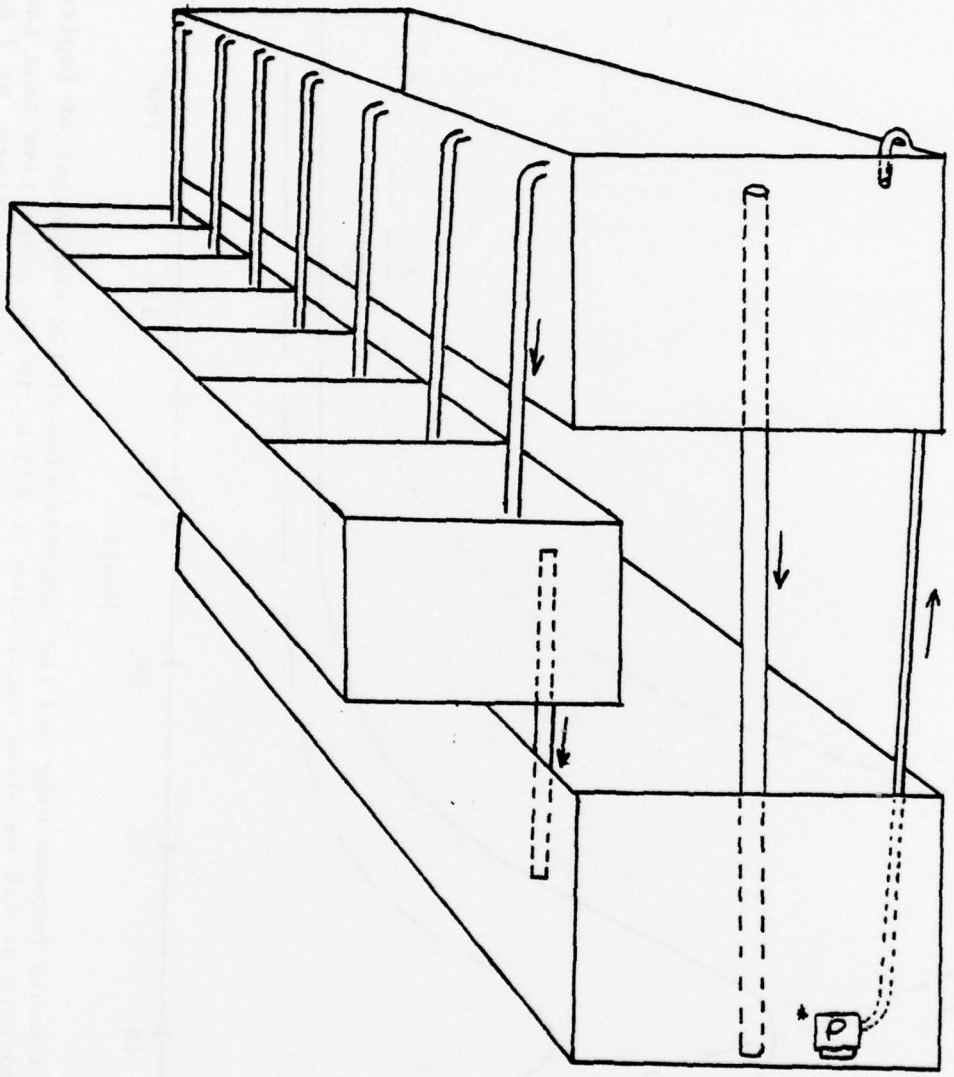

Figure 1. Diagram of the large volume flow system used in the laboratory studies (* only one pump and Tygon tubing are shown for simplicity).

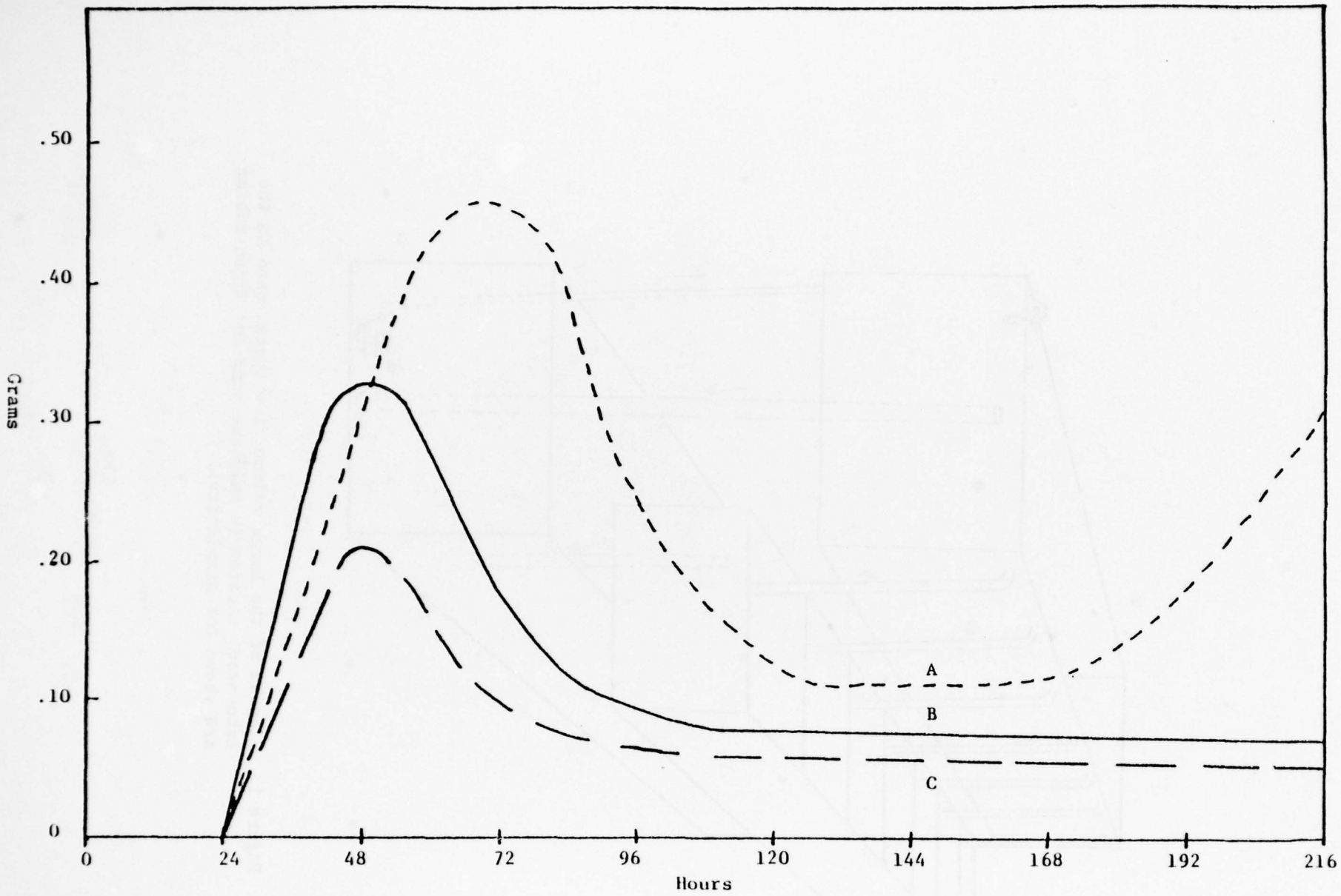

Figure 2. The relationship between time and the concentration of the components of Empire Mix crude oil which fluoresce at 418 nm after excitation at 403 nm in the respective test tanks. A, Mixing Tank (Tank A); B, Oil reservoir tank (Tank D); C, test tank B (average for 7 compartments).

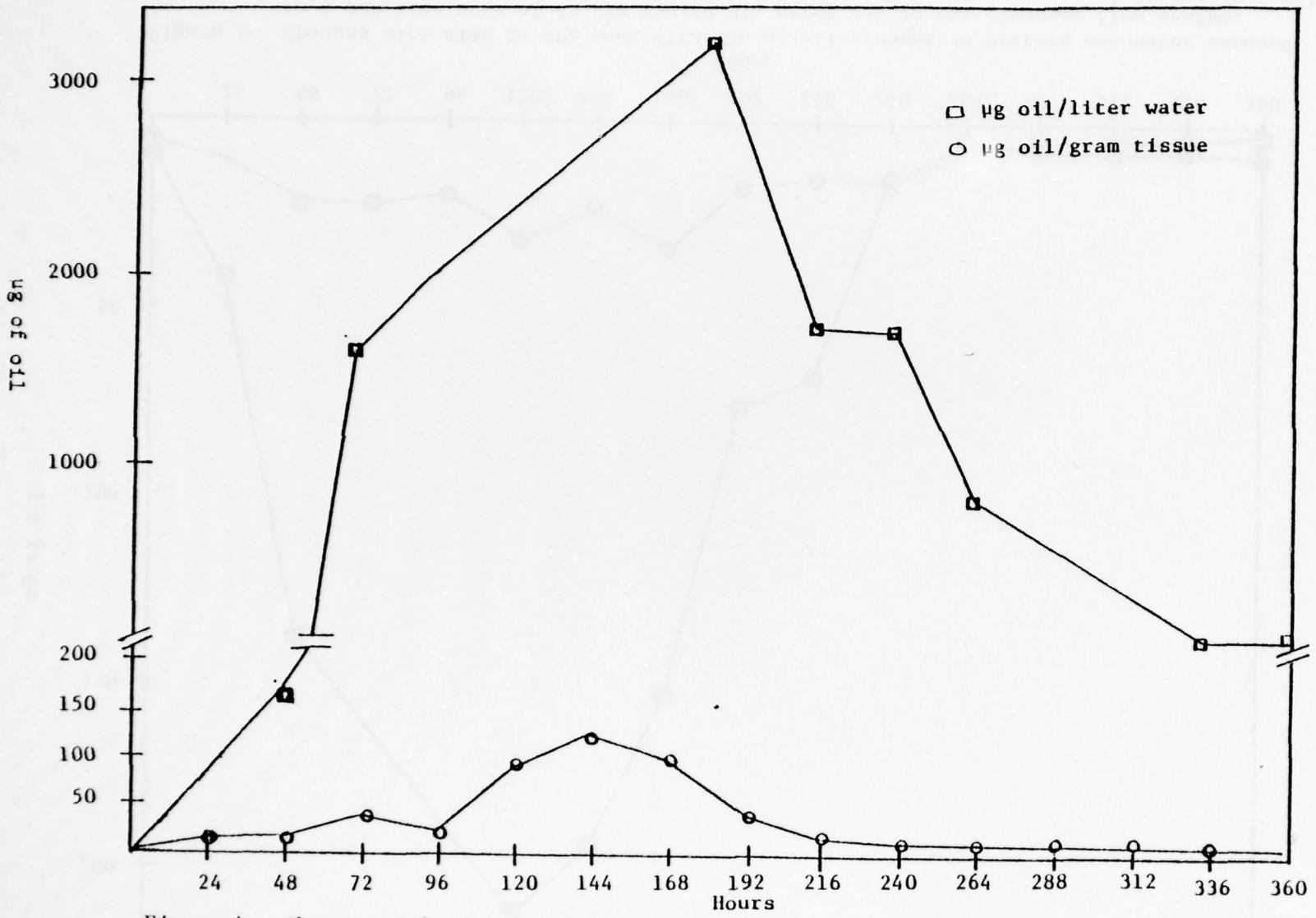

Figure 4. Changes with time in the concentration of oil present in oysters and water exposed to a one time dose of 75 ppm Empire Mix crude oil in a bioassay flow system. Each □ equals average of 11 water samples; each ○ equals average of 14 oyster samples.

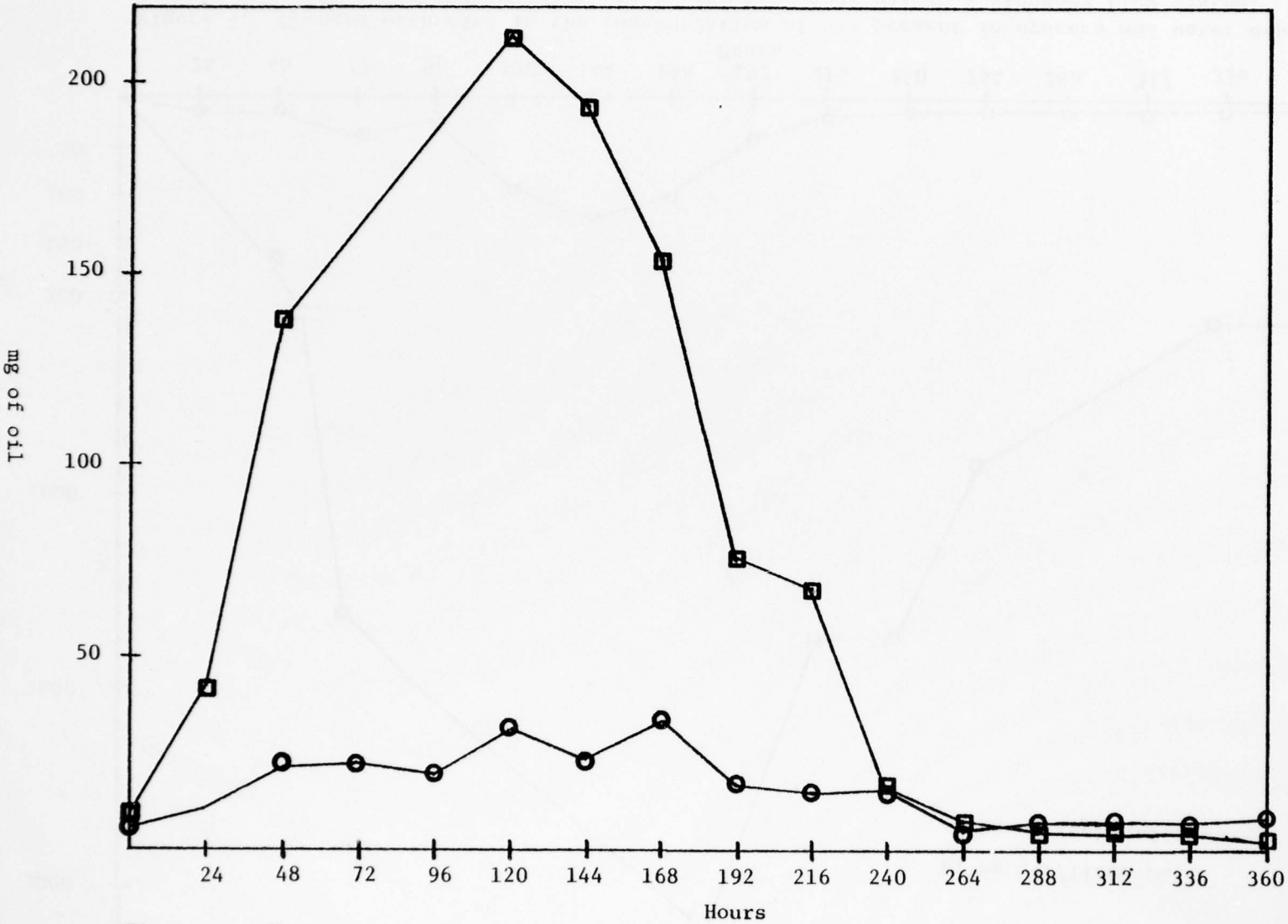

Figure 3. Changes with time in the concentration of oil present in oysters and water exposed to a one time dose of 25 ppm Empire Mix crude oil in our bioassay flow system. Each □ equals average of 11 water samples; each ○ equals average of 14 oyster samples.